

# Effect of oral nitroethane and 2-nitropropanol administration on methane-producing activity and volatile fatty acid production in the ovine rumen <sup>☆</sup>

R.C. Anderson <sup>a,\*</sup>, G.E. Carstens <sup>b</sup>, R.K. Miller <sup>b</sup>, T.R. Callaway <sup>a</sup>, C.L. Schultz <sup>a</sup>,  
T.S. Edrington <sup>a</sup>, R.B. Harvey <sup>a</sup>, D.J. Nisbet <sup>a</sup>

<sup>a</sup> United States Department of Agriculture, Agricultural Research Service, Food and Feed Safety Research Unit, 2881 F&B Road, College Station, TX 77845, USA

<sup>b</sup> Department of Animal Science, Texas A&M University, College Station, TX 778543, USA

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## Abstract

Strategies are sought to reduce economic and environmental costs associated with ruminant methane emissions. The effect of oral nitroethane or 2-nitropropanol administration on ruminal methane-producing activity and volatile fatty acid production was evaluated in mature ewes. Daily administration of 24 and 72 mg nitroethane/kg body weight reduced ( $P < 0.05$ ) methane-producing activity by as much as 45% and 69% respectively, when compared to control animals given no nitroethane. A daily dose of 120 mg 2-nitropropanol/kg body weight was needed to reduce ( $P < 0.05$ ) methane-producing activity by 37% from that of untreated control animals. Reductions in methane-producing activity may have been diminished by the last day (day 5) of treatment, presumably due to ruminal adaptation. Oral administration of nitroethane or 2-nitropropanol had little or no effect on accumulations or molar proportions of volatile fatty acids in ruminal contents collected from the sheep. These results demonstrate that nitroethane was superior to 2-nitropropanol as a methane inhibitor and that both nitrocompounds reduced ruminal methanogenesis *in vivo* without redirecting the flow of reductant generated during fermentation to propionate and butyrate.

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## 1. Introduction

Ruminal methane production is a digestive inefficiency resulting in the loss 2–4% of gross energy intake for animals consuming high concentrate diets and up to 15% gross energy intake for animals consuming high forage diets (Johnson and Johnson, 1995; Van Nevel and Demeyer,

1996). Methane is a greenhouse gas that has been implicated in contributing to global warming (Johnson and Johnson, 1995; Moss et al., 2000). In the United States, about 20% of the total methane output is produced from enteric fermentation, of which ruminants are the major producers (EPA, 2004).

Numerous chemical inhibitors have been shown to reduce methanogenesis but many of these also inhibited the oxidation of hydrogen (Van Nevel and Demeyer, 1995, 1996). Metabolic processes that consume hydrogen are thought to be beneficial to the animal and the microbial population because they prevent the accumulation of hydrogen to levels potentially inhibitory to ruminal fermentation (Miller, 1995). Moreover, the effects of many of these

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\* Corresponding author. Tel.: +1 979 260 9317; fax: +1 979 260 9332.  
E-mail address: [anderson@ffsru.tamu.edu](mailto:anderson@ffsru.tamu.edu) (R.C. Anderson).

previously studied inhibitors on methane production were often transient in nature, likely due to the ruminal ability to adapt to ecological challenges (Van Nevel and Demeyer, 1995).

Some more recent attempts to develop interventions to reduce ruminal methanogenesis have been successful either in prolonging the methane reducing potential in vivo (Kung et al., 2003; Machmüller and Kreuzer, 2004; Wright et al., 2004) or in conserving the energetic efficiencies associated with hydrogen-transfer reactions in vitro (Anderson and Rasmussen, 1998; Asanuma et al., 1999; Iwamoto et al., 2002; Sar et al., 2005). Other recent research efforts to inhibit methanogenesis have yielded varying degrees of success (Dohme et al., 2001; Fievez et al., 2003; Lee et al., 2002; Miller and Wolin, 2001; Ungerfeld et al., 2003).

Nitroethane and 2-nitropropanol have been shown to inhibit ruminal methanogenesis in vitro without markedly increasing hydrogen accumulations ( $<3\text{ }\mu\text{mol/ml}$  ruminal fluid) or adversely affecting the ratio of acetate to propionate produced in mixed culture incubations (Anderson et al., 2003). A particularly attractive feature of these nitro-compounds is that they also inhibit the growth of the zoonotic pathogens, *Listeria monocytogenes*, *Campylobacter* and *Salmonella* (Dimitrijevic et al., 2005; Jung et al., 2003, 2004a,b) and thus have the potential to be developed into a responsible preharvest food safety program. Presently, we evaluated the effect of oral administration of nitroethane and 2-nitropropanol on ruminal methane producing activity and volatile fatty acid production in vivo. Portions of this work were presented in preliminary form at the 10th International Symposium of Ruminant Physiology (Anderson et al., 2004).

## 2. Methods

### 2.1. Experimental design

Thirty mature ewes averaging  $66.3 \pm 13.1$  (SD) kg and maintained on a Bermudagrass hay:cracked corn diet (9:1) with ad libitum access to mineral supplementation were randomly allocated ( $n=5$  per treatment) to 0, 24 or 72 mg nitroethane/kg body weight per day (experiment 1) or to 0, 40 or 120 mg 2-nitropropanol/kg body weight per day (experiment 2) for five consecutive days. Treatments were administered via oral gavage of a sodium salt solution of nitroethane (Majak et al., 1986) diluted with water to 120 mg nitroethane/ml, or an aqueous solution containing 400 mg 2-nitropropanol/ml. These solutions were further diluted with water in order to administer approximately equal volumes to all sheep within each experiment. Each day's treatments were administered in two equal sized portions given at the morning (08:00) and afternoon (16:00) meals. Ruminal fluid was collected via stomach tube 2 h after administration of the morning meal 3 days before initiation of treatment (pretreatment) and on days 1, 2 and 5 of treatment. The ruminal fluid was transferred immediately upon collection into serum vials which were then

capped and maintained at ambient temperature (approximately 20–30 °C) until returned to the lab (within 1 h) for determinations of volatile fatty acid concentrations and methane-producing activity. Each serum vial was filled completely (approximately 60 ml) before capping to minimize contact of air with the fluid.

### 2.2. Analytical

Volatile fatty acid concentrations were measured by gas chromatography (Hinton et al., 1990). Methane-producing activity was determined by in vitro incubation of 5 ml ruminal fluid with 5 ml anaerobic dilution solution (Bryant and Burkey, 1953) containing 60 mM sodium formate and 0.2 g finely ground alfalfa (to pass a 4 mm screen). The tubes were capped and incubated 3 h at 39 °C under a hydrogen:carbon dioxide (50:50 mix) atmosphere. At the end of the incubation period, methane concentration present in the headspace of the incubations was determined by gas chromatography (Allison et al., 1992).

### 2.3. Statistical analysis

Tests for effects of treatment were performed by a repeated measures analysis of variance and LSD All-pair-wise Multiple Comparisons of means (Statistix® 8 Analytical Software, Tallahassee, FL, USA). Resultant critical values for comparison (CVC) are presented and are the minimum difference between two means to achieve significance at  $P < 0.05$ .

## 3. Results

Nitroethane treatment reduced methane-producing activity in ruminal contents collected from sheep administered 24 or 72 mg nitroethane/kg body weight per day when compared to control sheep given no nitroethane (Table 1). A main effect of day of treatment and a treatment  $\times$  day of treatment interaction were also observed, due primarily to a slight rebounding in methane-producing activities measured in ruminal fluid collected from treated animals on day 5 of treatment (Table 1).

Main effects of nitroethane treatment or a treatment  $\times$  day of treatment interaction ( $P > 0.05$ ) were not observed on ruminal concentrations of acetate, propionate and butyrate (Fig. 1). A main effect of day of treatment ( $P \leq 0.0017$ ) was observed on concentrations of these volatile fatty acids as concentrations were lowest in contents collected pre-treatment (Fig. 1).

Main effects of 2-nitropropanol treatment and day of treatment on ruminal methane-producing activity were not observed (Table 2); however, a significant treatment  $\times$  day of treatment interaction was observed, due primarily to lower rates of methane production measured from ruminal contents collected from sheep administered 120 mg 2-nitropropanol/kg body weight per day, especially on day 2 of treatment (Table 2).

Table 1  
Effect of oral nitroethane administration on methane-producing activity in ovine rumen contents in vivo

Treatment (per kg body weight day <sup>-1</sup> ) <sup>a</sup>	Mean (±SD) methane-producing activity (μmol CH <sub>4</sub> /g rumen fluid per hour)			
	Pretreatment	Day 1	Day 2	Day 5
0 mg nitroethane	6.76 ± 1.0 <sup>c,g</sup>	5.66 ± 1.1 <sup>d,g</sup>	6.61 ± 1.0 <sup>c,g</sup>	6.16 ± 0.6 <sup>c,d,g</sup>
24 mg nitroethane	6.79 ± 0.3 <sup>c,g</sup>	4.25 ± 0.8 <sup>e,h</sup>	3.60 ± 0.9 <sup>e,h</sup>	5.10 ± 0.6 <sup>d,h</sup>
72 mg nitroethane	6.82 ± 0.3 <sup>c,g</sup>	3.25 ± 0.6 <sup>e,i</sup>	2.00 ± 0.5 <sup>f,i</sup>	4.24 ± 0.7 <sup>d,h</sup>
Treatment effect		$P = 0.0001$		
Day effect		$P < 0.0001$		
Interaction		$P < 0.0001$		
CVC <sup>b</sup> for same level of treatment		0.73		
CVC <sup>b</sup> for different level of treatment		0.97		

<sup>a</sup> Administered via oral gavage ( $n = 5$  per treatment) of the sodium salt of nitroethane in two equal sized portions at 08:00 and 16:00. Data were analyzed by a repeated measures analysis of variance and LSD separation of means.

<sup>b</sup> CVC, critical value for comparison of means (the minimum difference between two means to achieve significance at  $P < 0.05$ ).

<sup>c,d,e,f</sup> Means within treatment (columns) with unlike superscripts differ ( $P < 0.05$ ).

<sup>g,h,i</sup> Means between treatments (rows) with unlike superscripts differ ( $P < 0.05$ ).

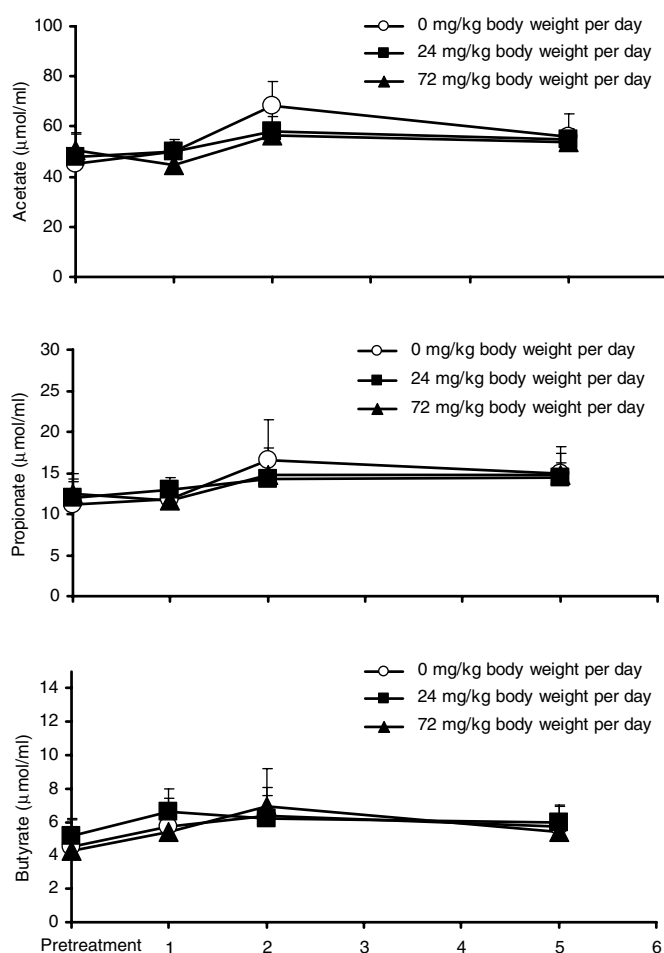


Fig. 1. Effect of oral nitroethane administration on ovine ruminal volatile fatty acid concentrations. Treatments were administered via oral gavage ( $n = 5$  per treatment) of the sodium salt of nitroethane in two equal sized portions at 08:00 and 16:00. Data were analyzed by a repeated measures analysis of variance and LSD separation of means. Critical value for comparison (the minimum difference between two means to achieve significance at  $P < 0.05$ ) for the same level of treatment were 8.56, 3.33 and 1.46 μmol/ml for acetate, propionate and butyrate, respectively; critical value for comparison of means  $P < 0.05$  for different level of treatment were 9.05, 3.59 and 1.92 μmol/ml, respectively.

A main effect of 2-nitropropanol treatment ( $P = 0.0096$ ) was observed on ruminal acetate concentrations, with the overall average concentration ( $36.54 \pm 10.74$  μmol/ml) measured in contents collected from sheep treated with 120 mg 2-nitropropanol/kg body weight per day being 13.6% and 16.3% lower than average concentrations measured in sheep administered 40 or 0 mg 2-nitropropanol/kg body weight per day, respectively. A main effect of day of treatment ( $P < 0.0001$ ) was observed on ruminal acetate, propionate and butyrate concentrations, due mainly to higher concentrations in contents collected on days 2 and 5 of treatment (Fig. 2).

Oral nitroethane or 2-nitropropanol administration did not cause any observable adverse physiologic or behavioral effects on the sheep.

#### 4. Discussion

Consistent with earlier in vitro observations (Anderson et al., 2003), results presented here confirm the methane-inhibiting properties of both nitroethane and 2-nitropropanol. Our results showed that nitroethane is the superior methane-inhibiting compound, with daily oral doses of 24 and 72 mg nitroethane/kg body weight causing reductions in methane-producing activity by as much as 45% and 69% respectively, when compared to control animals given no nitroethane (Table 1). In contrast, a daily oral dose of 120 mg 2-nitropropanol/kg body weight was needed to reduce methane-producing activity by 38% from that of untreated control animals (Table 2). Reductions in methane-producing activity observed on day 2 of nitrocompound treatment suggest that the effect of both nitrocompounds was dose dependent. Reductions in methane-producing activity may have been diminished by day 5 of treatment indicating a potential adaptation of the ruminal microflora of the nitrocompounds (Tables 1 and 2).

The propensity of the rumen ecosystem to adapt to a variety of methane inhibiting compounds has long been recognized (McAllister et al., 1996; Van Nevel and Demeyer, 1996). In the present case, ruminal adaptation to the

Table 2  
Effect of oral 2-nitropropanol administration on methane-producing activity in ovine rumen contents in vivo

Treatment (per kg body weight day <sup>-1</sup> ) <sup>a</sup>	Mean (±SD) methane-producing activity (μmol CH <sub>4</sub> /g rumen fluid per hour)			
	Pretreatment	Day 1	Day 2	Day 5
0 mg 2-nitropropanol	6.18 ± 1.0 <sup>c,e</sup>	6.95 ± 1.4 <sup>c,e</sup>	7.00 ± 0.8 <sup>c,e</sup>	6.40 ± 0.9 <sup>c,e</sup>
40 mg 2-nitropropanol	7.29 ± 1.7 <sup>c,e</sup>	6.12 ± 0.7 <sup>d,e</sup>	6.03 ± 0.9 <sup>d,e</sup>	6.61 ± 1.5 <sup>c,d,e</sup>
120 mg 2-nitropropanol	6.02 ± 1.3 <sup>c,e</sup>	6.24 ± 0.8 <sup>c,e</sup>	4.37 ± 0.9 <sup>d,f</sup>	5.77 ± 0.7 <sup>c,e</sup>
Treatment effect	$P = 0.1432$			
Day effect	$P = 0.0942$			
Interaction	$P = 0.0086$			
CVC <sup>b</sup> for the same level of treatment	1.04			
CVC <sup>b</sup> for different level of treatment	1.46			

<sup>a</sup> Administered via oral gavage ( $n = 5$  per treatment) of an aqueous solution of 2-nitropropanol in two equal sized portions at 08:00 and 16:00. Data were analyzed by a repeated measures analysis of variance and LSD separation of means.

<sup>b</sup> CVC, critical value for comparison of means (the minimum difference between two means to achieve significance at  $P < 0.05$ ).

<sup>c,d</sup> Means within treatment (columns) with unlike superscripts differ ( $P < 0.05$ ).

<sup>e,f</sup> Means between treatments (rows) with unlike superscripts differ ( $P < 0.05$ ).

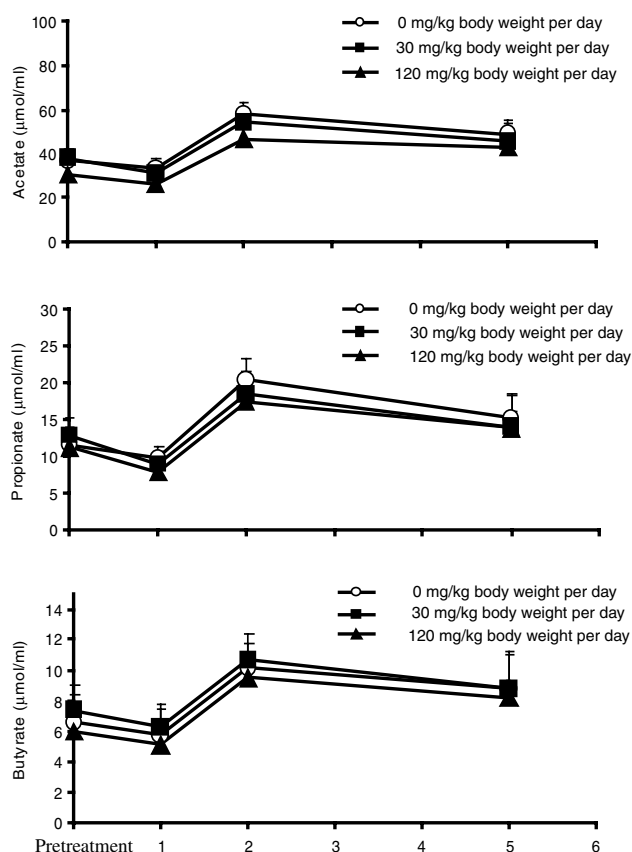


Fig. 2. Effect of oral 2-nitropropanol administration on ovine ruminal volatile fatty acid concentrations. Treatments were administered via oral gavage ( $n = 5$  per treatment) of an aqueous solution of 2-nitropropanol in two equal sized portions at 08:00 and 16:00. Data were analyzed by a repeated measures analysis of variance and LSD separation of means. Critical value for comparison (the minimum difference between two means to achieve significance at  $P < 0.05$ ) for the same level of treatment were 7.23, 2.81 and 1.78 μmol/ml for acetate, propionate and butyrate, respectively; critical value for comparison of means  $P < 0.05$  for different level of treatment were 7.80, 3.69 and 2.21 μmol/ml, respectively.

nitrocompounds may have been due to an in vivo enrichment of nitrocompound-reducing bacteria capable of deplet-

ing effective concentrations of the inhibitors. Although this hypothesis has yet to be confirmed, at least one ruminal microbe, *Denitrobacterium detoxificans*, an obligate respiring anaerobe, is known to metabolize nitroethane and a variety of other nitrocompounds, coupling its reduction to the oxidation of hydrogen or formate (Anderson et al., 2000). While typically present at low concentrations ( $\leq 10^3$  organisms/ml), populations of this bacterium were enriched during growth with increasing concentrations of nitrocompound (Anderson et al., 1996). Moreover, addition of *D. detoxificans* and an appropriate amount of a suitable electron acceptor (20 mM nitrate) to unadapted mixed ruminal fluid incubations resulted in >94% reduction in methane production and significant increases in rates of nitrate disappearance (Anderson et al., 1998). Our observations that these and other related nitrocompounds (Anderson et al., 1998, 2003) exerted an immediate effect on methanogenesis in non-adapted populations indicates that they act primarily to directly inhibit methanogenesis rather than solely serving as alternative electron acceptors. In the present experiment, for instance, the amount of reductant possibly consumed by the reduction of the highest administrations of the nitrocompounds, if acting solely as terminal electron acceptors, accounts for <5% the amount of methane (30 g/day) reasonably expected to be produced by sheep of this size (Pinares-Patiño et al., 2003). In contrast to what occurs in nonadapted populations, competitive consumption of reductant by nitro-reducing bacteria could possibly play a role in reducing enteric methanogenesis in adapted populations provided sufficient amounts of either acceptor could be safely administered to the animals. Whereas we observed no adverse effect of either nitrocompound on the sheep in this study, upper limits to be safely administered have yet to be determined.

In agreement with earlier in vitro results (Anderson et al., 2003), nitroethane had no effect on amounts or molar proportions of volatile fatty acids produced (Fig. 1). Whereas 2-nitropropanol caused slight reductions in ruminal acetate accumulations, it did not increase accumulations of



propionate or butyrate (Fig. 2). Thus, neither nitrocompound caused a redirection of reductant (electrons) that otherwise would have been consumed during the reduction of carbon dioxide to methane to be used for the production of the more reduced volatile fatty acids, propionate and butyrate. Some other methane inhibiting chemicals, such as monensin (Mbanzamihiho et al., 1996) and 9,10 anthraquinone (Kung et al., 2003), typically cause decreased production of acetate and increased production of propionate within ruminal contents. The fate of the unused electrons in the present study is not yet known but further studies are warranted to determine if these may have been consumed by anabolic cell processes (i.e., microbial cell growth) or by other reductive processes that may benefit the host.

## 5. Conclusions

Results presented here are the first to confirm the methane-inhibiting activity of nitroethane and 2-nitropropanol in vivo and showed that nitroethane had greater methane-inhibiting activity than 2-nitropropanol. The methane-inhibiting activity of the nitrocompounds appeared to be transient, probably due to an adaptation occurring within the rumen, which possibly could be resolved by increasing the amount of nitrocompound administered. Our findings that the nitrocompounds had little, if any, effect on amounts or molar proportions of volatile fatty acids produced within the rumen indicates that fermentative efficiencies associated with microbial interspecies hydrogen transfer were not compromised. Unlike most recent attempts to develop interventions to reduce ruminal methanogenesis, the nitrocompounds have the advantage in that they have also been shown to inhibit the growth of certain zoonotic pathogens. Further in vivo work is needed to determine if nitro-inhibition can be developed into a viable ruminal methane-reducing strategy having the potential to concomitantly reduce the carriage of certain zoonotic pathogens.

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## References

- Allison, M.J., Mayberry, W.R., McSweeney, C.S., Stahl, D.A., 1992. *Synergistes jonesii*, gen. nov., sp. nov.: a ruminal bacterium that degrades toxic pyridinediols. Syst. Appl. Microbiol. 15, 522–529.
- Anderson, R.C., Rasmussen, M.A., 1998. Use of a novel nitrotoxin-metabolizing bacterium to reduce ruminal methane production. Bioresour. Technol. 64, 89–95.
- Anderson, R.C., Rasmussen, M.A., Allison, M.J., 1996. Enrichment and isolation of a nitropropanol-metabolizing bacterium from the rumen. Appl. Environ. Microbiol. 62, 3885–3886.
- Anderson, R.C., Rasmussen, M.A., Jensen, N.S., Allison, M.J., 2000. *Denitrobacterium detoxificans* gen. nov., sp. nov., a ruminal bacterium that respire on nitrocompounds. Int. J. System. Evol. Microbiol. 50, 633–638.
- Anderson, R.C., Callaway, T.R., Van Kessel, J.S., Jung, Y.S., Edrington, T.S., Nisbet, D.J., 2003. Effect of select nitrocompounds on ruminal fermentation; an initial look at their potential to reduce economic and environmental costs associated with ruminal methanogenesis. Bioresour. Technol. 90, 59–63.
- Anderson, R.C., Carstens, G.E., Miller, R.K., Callaway, T.R., Schultz, C.L., Edrington, T.S., Harvey, R.B., Nisbet, D.J., 2004. Effect of nitroethane administration on ruminal VFA production and specific activity of methane production. J. Animal Feed Sci. 13 (Suppl. 1), 23–26.
- Asanuma, N., Iwamoto, M., Hino, T., 1999. Effect of the addition of fumarate on methane production by ruminal microbes in vitro. J. Dairy Sci. 82, 780–787.
- Bryant, M.P., Burkey, L.A., 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. 36, 205–217.
- Dimitrijevic, M., Anderson, R.C., Callaway, T.R., Nisbet, D.J., 2005. Inhibitory effect of select nitroalkanes on growth rate of *Listeria monocytogenes* in vitro. Abstracts of 1st International Conference Environmental Industrial and Applied Microbiology, Badajoz, Spain, March 15–18.
- Dohme, F., Machmüller, A., Kreuzer, M., 2001. Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. Lett. Appl. Microbiol. 32, 47–51.
- EPA, 2004. US Emissions Inventory, 2004. <<http://yosemite.epa.gov/OAR/globalwarming.nsf/content/ResourceCenterPublicationsGHGEmissionsUSEmissionsInventory2004.html>>. (Accessed 15.10.04).
- Fievez, V., Dohme, F., Danneels, M., Raes, K., Demeyer, D., 2003. Fish oils as potent rumen methane inhibitors and associated effects on rumen fermentation in vitro and in vivo. Animal Feed Sci. Technol. 104, 41–58.
- Hinton, A., Corrier, D.E., Spates, G.E., Norman, J.O., Ziprin, R.L., Beier, R.C., DeLoach, J.R., 1990. Biological control of *Salmonella typhimurium* in young chickens. Avian Dis. 34, 626–633.
- Iwamoto, M., Asanuma, N., Hino, T., 2002. Ability of *Selenomonas ruminantium*, *Veillonella parvula*, and *Wolinella succinogenes* to reduce nitrate and nitrite with special reference to the suppression of ruminal methanogenesis. Anaerobe 8, 209–215.
- Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. J. Animal Sci. 73, 2483–2492.
- Jung, Y.S., Anderson, R.C., Genovese, K.J., Edrington, T.S., Callaway, T.R., Byrd, J.A., Bischoff, K.M., Harvey, R.B., McReynolds, J., Nisbet, D.J., 2003. Reduction of *Campylobacter* and *Salmonella* in pigs treated with a select nitrocompound. In: Proceedings of the 5th international symposium on the epidemiology and control of foodborne pathogens in pork, Hersonissos, Crete, pp. 205–207.
- Jung, Y.S., Anderson, R.C., Callaway, T.R., Edrington, T.S., Genovese, K.J., Harvey, R.B., Poole, T.L., Nisbet, D.J., 2004a. Inhibitory activity of 2-nitropropanol against select foodborne pathogens in vitro. Lett. Appl. Microbiol. 39, 471–476.
- Jung, Y.S., Anderson, R.C., Edrington, T.S., Genovese, K.J., Byrd, J.A., Callaway, T.R., Nisbet, D.J., 2004b. Experimental use of 2-nitropropanol for reduction of *Salmonella typhimurium* in the ceca of broiler chicks. J. Food Prot. 67, 1945–1947.
- Kung, L., Smith, K.A., Smagala, A.M., Endres, K.M., Bessett, C.A., Ranjit, N.K., Yaissle, J., 2003. Effects of 9,10 anthraquinone on ruminal fermentation, total-tract digestion, and blood metabolite concentrations in sheep. J. Animal Sci. 81, 323–328.
- Lee, S.S., Hsu, J., Mantovani, H.C., Russell, J.B., 2002. The effect of bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5, on ruminal methane production in vitro. FEMS Microbiol. Lett. 217, 51–55.
- Machmüller, A., Kreuzer, M., 2004. Impact of the methane-suppressing feed additive myristic acid on energy and calcium balance of sheep. J. Animal Feed Sci. 13 (Suppl. 1), 151–154.
- Majak, W., Cheng, K.-J., Hall, J.W., 1986. Enhanced degradation of 3-nitropropanol by ruminal microorganisms. J. Animal Sci. 62, 1072–1080.

- Mbanzamihiho, L., van Nevel, C.J., Demeyer, D.I., 1996. Lasting effects of monensin on rumen and caecal fermentation in sheep fed a high grain diet. *Animal Feed Sci. Technol.* 62, 215–228.
- McAllister, T.A., Okine, E.K., Mathison, G.W., Cheng, K.-J., 1996. Dietary, environmental and microbiological aspects of methane production in ruminants. *Can. J. Animal Sci.* 76, 231–243.
- Miller, T.L., 1995. The ecology of methane production and hydrogen sinks in the rumen. In: Englehardt, W.V., Leonhard-Marek, S., Breves, G., Giesecke, D. (Eds.), *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*. Ferdinand Enke Verlag, Berlin, pp. 317–331.
- Miller, T.L., Wolin, M.J., 2001. Inhibition of growth of methane-producing bacteria of the ruminant forestomach by hydroxymethylglutaryl-SCoA reductase inhibitors. *J. Dairy Sci.* 84, 1445–1448.
- Moss, A.R., Jouany, J., Newbold, N., 2000. Methane production by ruminants: its contribution to global warming. *Ann. Zootechnol.* 49, 231–253.
- Pinares-Patiño, C.S., Ulyatt, M.J., Lassey, K.R., Barry, T.N., Holmes, C.W., 2003. Persistence of differences between sheep in methane emission under generous grazing conditions. *J. Agric. Sci.* 140, 227–233.
- Sar, C., Mwenya, B., Santoso, B., Takaura, K., Morikawa, R., Isogai, N., Asakura, Y., Toride, Y., Takahashi, J., 2005. Effect of *Escherichia coli* wildtype or its derivative with high nitrite reductase activity on in vitro ruminal methanogenesis and nitrate/nitrite reduction. *J. Animal Sci.* 83, 644–652.
- Ungerfeld, E.M., Rust, S.R., Burnett, R., 2003. Use of some novel alternative electron sinks to inhibit ruminal methanogenesis. *Reprod. Nutr. Dev.* 43, 189–202.
- Van Nevel, C., Demeyer, D., 1995. Feed additives and other interventions for decreasing methane emissions. In: Wallace, R.J., Chesson, A. (Eds.), *Biotechnology in Animal Feeds and Animal Feeding*. VCH Publishers, New York, pp. 329–349.
- Van Nevel, C.J., Demeyer, D.I., 1996. Control of rumen methanogenesis. *Environ. Monitor. Assess.* 42, 73–97.
- Wright, A.D.G., Kennedy, P., O'Neill, C.J., Toovey, A.F., Popovski, S., Rea, S.M., Pimm, C.L., Klein, L., 2004. Reducing methane emissions in sheep by immunization against rumen methanogens. *Vaccine* 22, 3976–3985.